Ratio Analysis of Reflectance Spectra (RARS): An Algorithm for the Remote Estimation of the Concentrations of Chlorophyll A, Chlorophyll B, and Carotenoids in Soybean Leaves

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An algorithm utilizing reflectance spectra bands in the photosynthetically active radiation (PAR) region of the solar spectrum was developed for the remote estimation of the concentrations of chlorophyll a, chlorophyll b, and carotenoids in soybeans. The defining of specific bands in the reflectance spectrum that corresponded to absorption bands of the individual pigments was basic to the development of the algorithm. The detection of these bands was rendered difficult by the lack of detail in reflectance spectra. It was therefore necessary to manipulate the reflectance spectra so that absorption bands due to specific pigments could be detected and their spectral maxima defined. It was found that by dividing soybean reflectance spectra by an arbitrarily selected reference soybean reflectance spectrum, ratio spectra were obtained in which the absorption bands could be distinctly seen and their wavelength defined. These ratio spectra allowed the defining of those bands corresponding to the absorption bands of chlorophyll a,

chlorophyll b, and carotenoids. The strong linear relationships of certain combinations of the bands in the ratio spectra to the concentrations of the photosynthetic pigments made it possible to develop a ratio analysis of reflectance spectra algorithm (RARS) by which the concentrations of these pigments could be calculated from the reflectance spectra. The measurements necessary for the development of RARS were made using soybeans which were grown at different nitrogen levels in order to obtain a range of reflectance spectra. A test of the RARS algorithm using other soybean plants showed very good agreement between measured pigment values and those calculated using RARS.

INTRODUCTION

The reflectance spectra of vegetation in the photosynthetically active radiation (PAR) region of the electromagnetic spectrum is the manifestation of the light absorption maxima of the different plant constituents. The pigments making the greatest contribution to light absorption in this region are those which are intimately involved in photosynthesis, namely, chlorophyll a and b, and the carot-

enoids. The absolute and relative concentrations of these photosynthetic pigments dictate the photosynthetic potential of the plant, that is, its efficiency in the utilization of PAR for biosynthetic purposes. It thus follows that changes in the concentrations of these pigments would relate strongly to the physiological status of the plant. In addition, the fraction of absorbed PAR (f_{apar}) at the canopy level is a function of the sum of the concentrations, and more precisely the molar extinctions, of chlorophyll *a*, chlorophyll *b*, and the carotenoids. Thus the estimation of individual pigment concentrations would permit f_{apar} to be expressed as a function of the concentration of the individual pigments.

The procedures commonly used in the determination of chlorophyll are destructive chemical techniques which involve the extraction of chlorophyll from leaf matter with organic solvents, followed by a spectrophotometric assay of chlorophyll (Arnon, 1969; Hiscox and Irealstam, 1979). Nondestructive techniques for the determination of total chlorophyll have been described (Benedict and Swidler, 1961; Inada, 1964; Takano and Tsundo, 1970; Wallihan, 1973; Hardwick and Baker, 1973; Macnicol et al., 1976). These techniques all involve relating the leaf reflectance at about 675 nm to the concentration of the total chlorophyll. A negative logarithmic correlation was found between the level of reflectance in the spectral region where reflectance was minimal (absorption maximal) and the concentration of chlorophyll. The low level of reflectance in this region gave rise to considerable variability among replicate samples which necessitated the use of large numbers of replications. Thomas and Gaussman (1977) observed a better correlation between the reflectance at 550 nm and the chlorophyll concentration than the correlation between reflectance at 675 nm and the chlorophyll concentration than that using the reflectance at 675 nm.

It was believed by the authors that reflectance spectra contained the necessary information for the development of algorithms for the remote sensitive determination of pigment concentrations which would be species independent. Upon looking at any representative reflectance spectrum, the absence of the detail necessary for any direct pigment analysis is quickly apparent. The high extinction coefficients of the chlorophylls and accessory pigments result in a very low re-

flectance in the PAR region, that is, 5% or less. This low reflectance coupled with the limited sensitivity and resolution of the radiometers commonly in use renders it extremely difficult to detect spectral bands which can be ascribed to the absorption of individual pigments. The detection and wavelength definition of these bands were required for the development of algorithms for the remote estimation of the concentrations of the photosynthetic pigments using reflectance spectra.

One means of amplifying small inflections in the reflectance spectra to detectable and resolvable levels involves dividing reflectance spectra by an arbitrarily selected reference reflectance spectrum. This results in a ratio spectrum which when multiplied by 100 is a percentage of the reference spectrum. This spectrum amplifies any absorbance differences between the two spectra at specific wavelengths. The use of the ratio spectra has allowed the identification of reflectance bands corresponding to the absorption bands of specific pigments. These bands were used in the development of a ratio analysis of reflectance spectra (RARS) algorithm, by which the concentrations of chlorophyll *a*, chlorophyll *b*, and the carotenoids can be estimated. The experiments necessary for the development and validation of this algorithm are described here.

EXPERIMENTAL METHODS

The investigations necessary for the development of the algorithm were conducted on greenhouse grown soybeans (*Glycine max.* Merr.). The plants were grown in perlite with the necessary complement of nutrients optimal for soybean growth, with the exception of nitrogen. The nitrogen (urea) concentration was varied between three groups of plants (10 plants in each group) to produce a range of physiological differences and reflectance spectra. The nitrogen (urea) concentrations used were $2 \times 10^{-3} M$, $1 \times 10^{-3} M$, and $5 \times 10^{-4} M$. These concentrations are considered to be high, medium, and low, respectively, for optimal soybean growth.

The plants were allowed to grow for six weeks after germination. The following measurements were then made: 1) rates of photosynthesis, 2) reflectance spectra of the soybean leaves, 3) ab-

sorption spectra of the intact leaf, extracts of the leaves, and solutions of pure chlorophyll *a*, chlorophyll *b*, and beta carotene, and 4) spectrophotometric determination of the concentrations of chlorophyll *a*, chlorophyll *b*, and the carotenoids after extraction from the soybean leaf.

Rate of Photosynthesis

The rates of photosynthesis were measured on the intact plant using two leaves of each plant in each nutrient group (60 replicates). These rates were determined using a LICOR Model 6200 infrared gas analyzer in the closed mode. The measurements were made in the laboratory with the light source consisting of a combination of water cooled low pressure sodium lamps and alkaline metal halide lamps operating at an intensity of approximately 1800 $\mu M / m^{-2} s^{-1}$. The ambient CO_2 concentration was 330 ppm. The temperature within the photosynthesis chamber did not exceed 27°C, while the relative humidity was approximately 58%. The CO_2 uptake measurements were made on a leaf area of 2.5 cm².

Reflectance Spectra

The reflectance spectra were determined using a LICOR Model 1800 integrating sphere radiometer with a resolution of 6 nm. The reflectance spectra were made on the same areas of the same leaves used in the photosynthesis measurements.

Spectrophotometric Determination of Pigment Concentrations

The photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) were extracted from the soybean leaves by placing the same portion (2.5 cm²) of the leaves used in the photosynthesis and reflectance measurements in 5 mL of dimethyl sulfoxide (DMSO) and extracting for 12 h in the dark (Hiscox and Isrealstam, 1979). One milliliter of the extract represents 0.25 cm² of the soybean leaf. The corresponding pure pigments (Sigma Chemicals, St. Louis, MO) were dissolved in DMSO at a concentration of 1×10^{-1} g / L.

The absorption spectra of the intact soybean leaves, DMSO extracts of the soybean leaves, pure chlorophyll *a*, pure chlorophyll *b*, pure β-carotene, and a mixture of the pure pigments were

determined using a double beam Perkin Elmer spectrophotometer with a resolution of 1 nm, and scanning from 300 to 750 nm at a speed of 60 nm / min. The absorption spectra of the extracts and pure pigments were determined with the extract in a quartz cuvette with a 10 mm light path. The absorption spectra of the intact leaf was determined with the leaf attached to the slit of the cuvette holder with the adaxial surface facing the light source. A leaf whose pigments had been removed with DMSO was used as a blank.

The concentrations of the extracted pigments were calculated from the absorbance values at 664 nm, 648 nm, and 470 nm using equations described by Lichtenthaler (1987), where

chlorophyll $a_c = 12.25A_{664\,nm} - 2.79A_{648\,nm}$,
chlorophyll $b_c = 21.50A_{648\,nm} - 5.10A_{664\,nm}$,
carotenoids$_c$ $= (1000A_{470\,nm} - 1.82\,chl\,a_c - 85.02$
$chl\,b_c) / 198$,

A = absorbance,
c = pigment concentration (μg/mL of extract).

These equations are valid only for DMSO extracts where chlorophyll *a*, chlorophyll *b*, and carotenoids have absorption maxima at 664 nm, 648 nm, and 470 nm, respectively.

RESULTS AND DISCUSSION

Reflectance Spectra

The mean ($n = 20$) reflectance spectra of each group of soybeans are shown in Figure 1. They show very little detail other than a maximum at approximately 550 nm and a minimum at approximately 675 nm, the regions of minimal and maximal chlorophyll absorption, respectively. It is only at these bands that significant differences in reflectance as the result of different nitrogen levels are seen.

Ratio Spectra

A technique which is frequently used in absorption spectrophotometry to detect small differences between spectra involves dividing one spectrum by another similar spectrum. This results in a ratio spectrum whereby any differences in absorbance at specific wavelengths are amplified to detectable levels. It also becomes possible to detect those bands at which absorbance due to
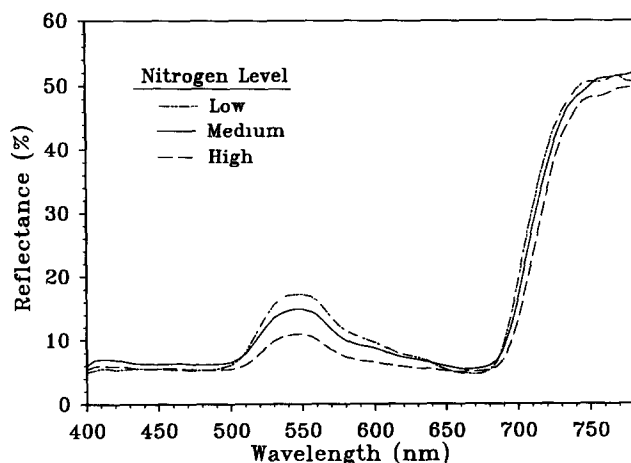
Figure 1. Mean reflectance spectra of soybeans grown at three nitrogen levels ($n = 20$ in each group). The nitrogen in the form of urea was present in the perlite growth matrix at concentrations of 2×10^{-3} M (high), 1×10^{-3} M (medium), and 5×10^{-4} M (low).

Figure 2. Ratio spectra obtained by dividing mean reflectance spectra of soybeans ($n = 20$) grown at high nitrogen level (reference spectra) by mean reflectance spectra of soybeans ($n = 20$) grown at medium nitrogen level.

specific chemical entities becomes minimal. It was believed that this technique could be successfully applied to reflectance spectra. A ratio spectrum obtained by dividing reflectance spectra by a reference reflectance spectrum should amplify differences between the spectra at specific bands due to absorption maxima and minima of the photosynthetic pigments.

The ratio spectrum necessary to establish the absorption maxima and minima of the soybean reflectance spectrum was obtained by dividing the reflectance spectra of the soybeans grown at a high nitrogen level (reference spectra) by the mean reflectance spectrum of the soybeans grown at a medium nitrogen level. The ratio spectrum obtained by dividing a spectrum with a lower overall reflectance by one with a higher overall reflectance results in a ratio spectrum where positive inflections are absorption maxima and negative inflections are absorption minima. The ratio spectrum was multiplied by 100 so that the ratio spectral values would be in terms of percentage of the reference spectrum.

The ratio spectrum as shown in Figure 2 has a number of inflection points, both positive and negative. It is thus apparent that reflectance spectra have considerable detail which is made apparent by the use of ratio spectra. The inflection bands of the ratio spectrum are manifestations of absorbance differences at the bands correspond-
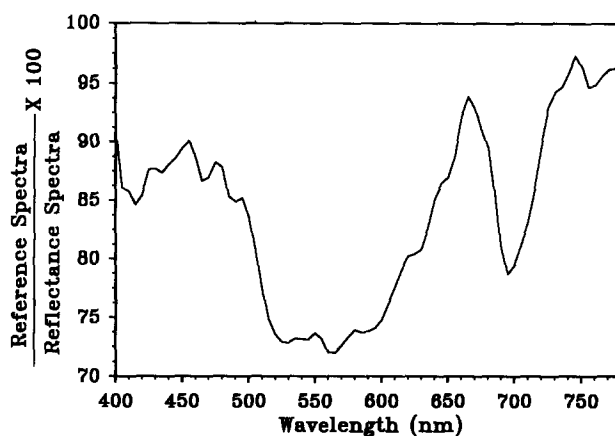
ing to the absorption maxima and minima of the photosynthetic pigments.

Pigment Absorption Band Identification

The absorption spectra of pure plant pigments (Fig. 3) were examined to relate the positive inflection bands of the ratio spectrum to the absorption maxima of specific pigments. The inflection band at 415 nm corresponds to convoluted absorption bands of chlorophyll *a* and *b*. The inflection bands at 460 nm and 650 nm coincide with two of the absorption bands of chlorophyll *b*. The inflection bands at 580 nm, 630 nm, and

Figure 3. Absorption spectra of pure plant pigments. These pigments were dissolved in DMSO at a concentration of 1×10^{-1} g/M. The solutions were kept in dark and refrigerated at $5°C$ prior to use.
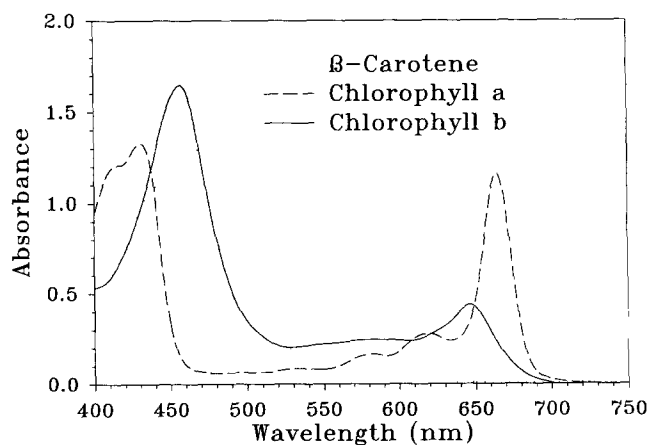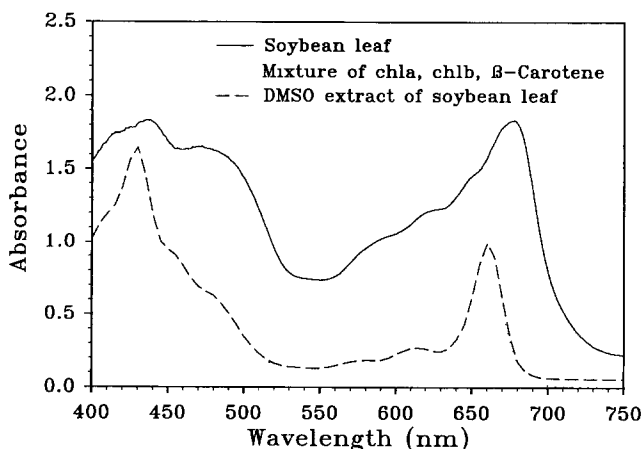
670 nm coincide with three of the absorption bands of chlorophyll *a*, while the inflections at 470 nm and 500 nm correspond to two of the absorption bands of beta carotene. The inflection at 750 nm is the point at which there is no further absorption by PAR and the slope toward increased reflectance is at a maximum.

All of the pure pigments had multiple absorption maxima as shown in Figure 3. The close proximity of the absorption bands results in considerable convolution of the spectrum containing a mixture of pigments. This convolution is seen in the absorption spectrum of a mixture of pure pigments, the DMSO extract of the soybean leaf, as well as the absorption spectrum of the intact leaf (Fig. 4). It should be noted that the chlorophyll *a* maximum in the red region undergoes a spectral shift when dissolved in DMSO.

Relation of Pigment Absorption Spectra to Action Spectra

The striking similarity of the spectra shown above, including the ratio spectrum (Fig. 2), the overlay of the pure pigments (Fig. 3), the mixture of the pure pigments and the intact leaf (Fig. 4) to the action spectrum of photosynthesis (Kok, 1965), emphasizes the dominant role of these compounds in photosynthesis. These pigments are the primary pigments contributing to the absorption of PAR, and thus to the reflectance spectrum in the PAR region.

Selection of Bands for Algorithm

The bands which were selected for the development of the algorithm for pigment concentration estimation included those with maxima at 675 nm, 650 nm, and 500 nm, which are the absorption bands of chlorophyll *a*, chlorophyll *b*, and the carotenoids, respectively. These pigment absorption bands were selected primarily because their maxima were least affected by convolution. The band at 700 nm of the ratio spectrum is the minima of chlorophyll *a* absorption, and the band at 750 nm is where there is no further absorption of PAR and the slope toward increased reflectance is at a maximum. It is at this point where reflectance becomes primarily a function of leaf structure and scatter.

The algorithms for pigment estimation were developed using ratio spectra, whereby the reflectance bands (60 soybean leaves) corresponding to the selected ratio bands or combinations of these bands were divided by these bands of the reference reflectance spectrum (mean spectra of the soybeans grown at a high nitrogen level).

It was found necessary to use combinations of ratio bands to minimize the effects of spectral convolution. The concentration of chlorophyll *a* had a strong linear relationship to the ratio, 675 nm / 700 nm of the ratio spectra (Fig. 5). It is believed that the significance of the band at 700 nm stems from its being the spectral point at which there is no further contribution to the reflectance spectra by chlorophyll *a*. The disap-

Figure 4. Absorption spectra of intact soybean leaf, DMSO extract of soybean leaf, and a mixture pure chlorophyll *a*, chlorophyll *b*, and beta carotene. The mixture was 1:1:1 of the pigments with each at a concentration of 1×10^{-1} g / L.

Figure 5. Ratio spectra band ratio at 675 nm / 700 nm versus chlorophyll *a* concentration (μg / mL). These are the band ratio and pigment values of 60 soybean leaves from plants grown at different nitrogen levels.
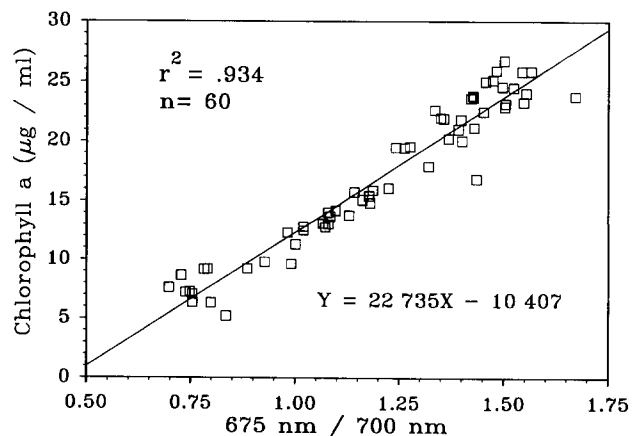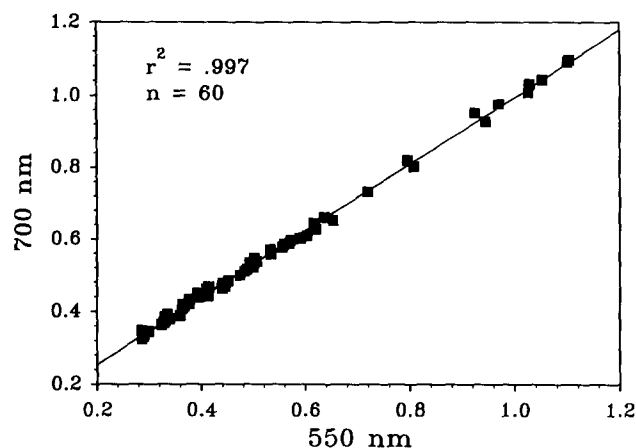
Figure 6. Reflectance band at 550 nm versus reflectance band at 700 nm. These are bands from spectra of 60 soybean leaves from plants grown at different nitrogen levels.

Figure 8. Ratio spectra band ratio at 760 nm / 500 nm versus carotenoid concentration (μg / mL). These are the band ratio and pigment values of 60 soybean leaves from plants grown at different nitrogen levels.

pearance of chlorophyll absorption at this reflectance band is borne out by its relationship to the reflectance band at 550 nm at which the absorbance of plant pigments is also minimal (Fig. 6). The concentration of chlorophyll *b* related best to 675 nm / (650 nm × 700 nm) as shown in Figure 7. The involvement of the chlorophyll *a* band in this ratio is a reflection of the convolution of the chlorophyll *a* and *b* absorption bands. The band ratio which best related to the carotenoid concentration was 760 nm / 500 nm (Fig. 8).

There was no band or combination of bands which related significantly to the rate of photosynthesis. The rate of photosynthesis was related best
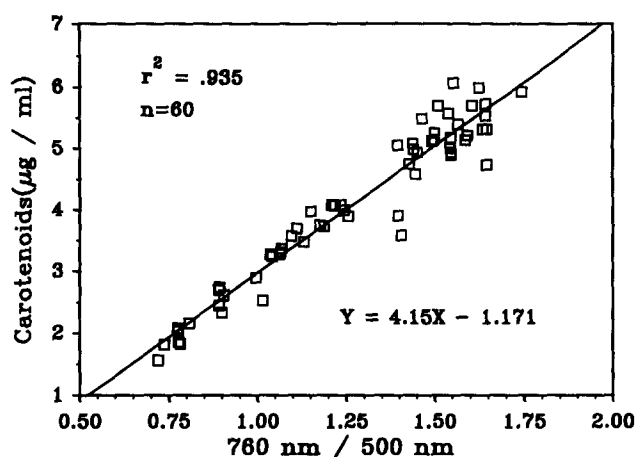
to the concentration of chlorophyll *a* (Fig. 9), where there was a low but significant correlation coefficient ($r^2 = 0.53$). The finding that only about 50% of changes in the rate of photosynthesis could be explained by the chlorophyll *a* concentration is not surprising as there are several factors other than the chlorophyll concentration which have an influence upon the rate of photosynthesis.

Development and Validation of RARS Algorithm

The equations for the RARS algorithm used in the estimation of pigment concentration were derived from the linear fit equations of the regression

Figure 7. Ratio spectra band ratio at 675 nm / (650 nm × 700 nm) versus chlorophyll *b* concentration (μg / mL).These are the band ratio and pigment values of 60 soybean leaves from plants grown at different nitrogen levels.

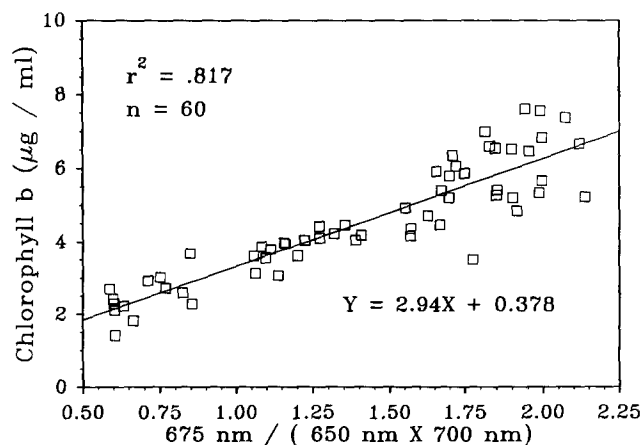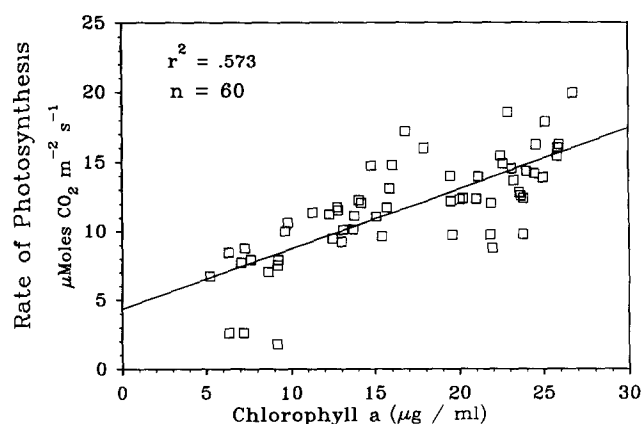*Figure 9.* The rates of photosynthesis versus the concentration of chlorophyll *a* (μg / mL). These are the rates of photosynthesis and the chlorophyll concentrations from 60 soybean leaves from plants grown at different nitrogen levels.

lines shown in Figures 5, 7, and 8. The derivation of these equations are given below.

Chlorophyll a equation:

$$\text{Linear fit} = 22.735X - 10.407$$

$$X = (S_{675}/S_{700})/(R_{675}/R_{700})$$

$$= S_{675}/S_{700} \times R_{700}/R_{675}$$

where $R_{700}/R_{675} = 3.101$, based on reflectance of reference spectrum. Thus,

$$X = S_{675} / S_{700} \times 3.101,$$

$$k_{chl\,a} = 3.101,$$

$$\text{chlorophyll } a \ (\mu g/mL) = 22.735 \times k_{chl\,a}(S_{675} / S_{700})$$
$$- 10.407,$$

S = reflectance spectrum for any soybean leaf,

R = reference reflectance spectrum.

Chlorophyll b equation:

$$\text{Linear fit} = 2.94X + 0.378,$$

$$X = (S_{675} / S_{650} \times S_{700}) \times (R_{650} \times R_{700} / R_{675}),$$

where $R_{650} \times R_{700} / R_{675} = 21.459$, based on reflectance of reference spectrum. Thus

$$X = (S_{675} / S_{650} \times S_{700})$$
$$\times 21.459,$$

$$k_{chl\,b} = 21.459,$$

$$\text{Chlorophyll } b \ (\mu g / mL) = 2.94 \times k_{chl\,b}(S_{675}/S_{650}$$
$$\times S_{700}) + 0.378,$$

S = reflectance spectrum for soybean leaf,

R = reference reluctance spectrum.

Carotenoid equation:

$$\text{Linear fit} = 4.145X - 1.171,$$

$$X = (S_{760} / S_{500}) / (R_{760} / R_{500})$$

$$= S_{760} / S_{500} \times R_{500} / R_{760},$$

where $R_{500} / R_{760} = 0.245$, based on reference spectrum. Thus

$$X = S_{760} / S_{500} \times 0.245,$$

$$k_{car} = 0.245,$$

$$\text{carotenoids } (\mu g / mL) = 4.14 \times k_{car}(S_{760} / S_{500})$$
$$- 1.171,$$

S = reflectance spectrum for any soybean leaf,

R = reference reflectance spectrum.

It should be noted ·that the value of k is dependent upon the reference reflectance spectrum used. The equations of the RARS algorithm allow the concentrations of the photosynthetic pigments to be determined directly from any reflectance spectra.

The RARS algorithm whose equations were derived above was tested using 10 soybean leaves selected at random from another group of plants. Reflectance spectra were taken, and the extracted pigment concentrations were determined. The pigment concentrations were also calculated using the appropriate equations and pigment constant. A comparison of the observed values and the calculated values are shown in Table 1. The measured values and the calculated values were in reasonably good agreement.

The RARS algorithm is ultimately based upon the specific wavelength maxima of the absorption bands of the pigments as well as their specific molar extinction coefficients. It can be safely assumed that these specific wavelength maxima and extinction coefficients remain the same for all vegetation regardless of species type. Although the carotenoid fraction is comprised of several related compounds with the carotenes and xanthophylls making up the bulk, we have found that they all have an absorption band with a maximum at 500 nm. Thus the RARS algorithm should be applicable to the estimation of the photosynthetic pigments of all higher plants. This must, of course, be verified with species other than soybeans.

It should be pointed out that although estimates of chlorophyll have been implied for many years in terms of green biomass, these studies

Table 1. A Comparison of the Concentrations of Chlorophyll *a*, Chlorophyll *b*, and the Carotenoids as Measured Spectrophotometrically, and Those Calculated Using the RARS Algorithm

Leaf No.	Chlorophyll a		Chlorophyll b		Carotenoids	
	Obs	Calcd	Obs	Calcd	Obs	Calcd
1	23.68	24 77	7.51	6.44	5.02	5 52
2	17.65	20 24	5.85	5.79	4.21	5 04
3	18 23	17 84	6.11	5.53	4 65	5.11
4	16.88	19 11	5.55	5 10	4 53	4.51
5	17.00	15.69	5 76	4 78	4.57	4 16
6	9.99	10 67	3.26	2 98	3 07	2.57
7	10.55	11.15	3.33	3.29	2.97	2 83
8	7 53	5 22	2 63	2.03	2 44	2.03
9	2.55	2.71	1.03	1 40	1 28	1.01
10	18.53	18.12	6.23	4 92	4 51	4 45

indicate that, for the first time, it may now become possible to remotely estimate the individual concentrations of chlorophyll *a* and *b*, and carotenoids.

The remote estimation of the absolute and relative concentrations of the photosynthetic pigments at the canopy level should permit an improved use of reflectance measurements for the assessment of plant status and development, as well as the evaluation of photosynthetic potential.

Significance of RARS in Measurement of F_{apar}

Another ramification of the remote estimation of the individual photosynthetic pigments is the relation to the fraction of PAR absorbed by vegetative canopies. The determination of f_{apar} has been the subject of study by a number of investigators including (Daughtry et al., 1983; Asrar et al., 1984; Fuchs et al., 1984; Gallo et al., 1985; Hall et al., 1990). The f_{apar}, as now measured and interpreted, does not weight f_{apar} throughout the PAR region according to the extinction coefficients of the pigment absorbing at a particular wavelength, but the assumption is implicit that the contribution of each photosynthetic pigment, on a molar basis, to the energetics of photosynthesis is the same.

The relative concentrations of the photosynthetic pigments should be expected to change as a function of a number of factors. These would include rate of biosynthesis, availability of substrate material, stage of growth, plant type, and environmental conditions. The concentration of chlorophyll *a* is the limiting factor in the utilization of light for photosynthesis, as it is the recipient of the energy absorbed by chlorophyll *b* and the carotenoids. Thus $(f_{apar})_1$, where the ratio of chlorophyll *a* to chlorophyll *b* and the carotenoids is "normal," has more photosynthetic potential than $(f_{apar})_2$, where the ratio of chlorophyll *a* to chlorophyll *b* and the carotenoids decreases, even if the f_{apar} values are the same. Conditions such as this can occur as the result of senescence (both normal and premature), certain nutrient deficiencies, and other stress conditions affecting the synthesis and degradation of the chlorophylls. Chlorophyll is converted to pheophytin when exposed to excessive light, low pH, and gases such as SO_2 and NO_2 during senescence (Lichtenthaler, 1987). This process is also a normal continuing

process in the plant but becomes accelerated under conditions of decreased pH and senescence. Chlorophyll upon conversion to pheophytin is no longer photosynthetically active, but continues to absorb PAR. However, the absorption maximum of pheophytin is shifted about 10 nm into the red relative to chlorophyll. Thus the contribution of pheophytin to the chlorophyll *a* would be minimal.

Studies are being planned to further assess the utility of RARS in an improved interpretation of f_{apar}.

CONCLUSIONS

The remote estimation of the photosynthetic pigments at the leaf level has been made possible by the development of an algorithm for the ratio analysis of reflectance spectra (RARS). The development of the algorithm was made possible by defining bands in the reflectance spectra which corresponded to the absorption bands of chlorophyll *a* and *b*, and the carotenoids. The detection and defining of absorption band maxima and minima were accomplished by dividing a reference soybean reflectance spectrum by a reflectance spectrum with a higher overall reflectance. A ratio spectrum was obtained which amplified spectral differences at bands related to the pigment absorption bands. A comparison of the ratio spectrum with the absorption spectrum of pure photosynthetic pigments allowed the defining of ratio inflection bands corresponding to the absorption bands of these pigments.

The RARS algorithm was developed using ratio spectra obtained by dividing soybean reflectance spectra by the reference soybean reflectance. The strong linear relationships of ratio spectra bands corresponding to pigment absorption bands, to the concentrations of specific photosynthetic pigments provided the basis for the development of equations and constants for the estimation of the concentration of these pigments in soybean leaves. It was now possible to estimate pigment concentrations using reflectance spectra. A good agreement was obtained when the pigment concentrations obtained by chemical analysis were compared with calculated values using RARS.

It should be stressed that these conclusions

are based solely upon results obtained with soybeans grown under varying nutrient nitrogen levels. RARS is based upon the absorption maxima of the photosynthetic pigments. These maxima are constant for all plant species, and as such, the algorithm should be compatible with any other species. However, the *k* values for the pigments will be a function of the reference spectrum.

The use of RARS at the canopy level could offer a means of accurately defining f_{apar} in terms of specific photosynthetically active pigment concentrations. Environmental stress conditions as manifested by decreases and relative changes in the concentrations of the photosynthetic pigments might be detected using RARS. Modifications of the RARS algorithm are being developed which may minimize the reflectance of soil backgrounds.

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